December 20, 2005

EPA/OPP MICROBIOLOGY LABORATORY
ESC, Ft. Meade, MD

Standard Operating Procedure
For

Biosafety Practices for Handling Select Agent Samples in Room D122

SOP Number: MB-18-00

U.S. Environmental Protection Agency

EPA/OPP MICROBIOLOGY LABORATORY
ESC, Ft. Meade, MD

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SOP Number: MB-18-00

Date Revised: 12-20-06

Initiated By: _____ Date: ___/___/___

Print Name:_____

Technical Review: _____ Date: ___/___/___

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Technical Staff

QA Review: _____ Date: ___/___/___

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QA Officer

Approved By: _____ Date: ___/___/___

Print Name:_____
Branch Chief

SHEM Review: _____ Date: ___/___/___

Print Name:_____
SHEM Manager

Effective Date: ___/___/___

Controlled Copy No.: _____

Withdrawn By: _____ Date: ___/___/___

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1.0 SCOPE AND APPLICATION:

1.1 The protocol outlines the required safety measures and procedures for working in all rooms associated with the Receiving Rooms D122, D122A and D123 during a Restricted Access Alert.

1.2 Additional procedures associated with this SOP can be found in SOP MB-01, Biosafety in the Laboratory, SOP QC-13, Performance Verification of Autoclaves and SOP MB-13, Handling Spills of Biohazardous Materials.

1.3 All manipulation of the Select Agent *Bacillus anthracis* will be conducted according to specifications identified in section 15.4 of SOP MB-01, Biosafety in the Laboratory. Any additional personal protective equipment (PPE) and procedures not identified in SOP MB-01, Biosafety in the Laboratory Section III, subsection 10.0, will apply as specified in the SOP.

1.4 This SOP may be amended as necessary to address additional Select Agents.

1.5 Room D122 may also be used for general biological laboratory work. Refer to SOP MB-01, Biosafety in the Laboratory, for procedures working under Biosafety Level (BSL) 1 and 2 conditions.

2.0 DEFINITIONS:

2.1 Bav = *Bacillus anthracis* (virulent strain) a Select Agent as defined by the CDC

2.2 BMBL = Biosafety in Microbiological and Biomedical Laboratories

2.3 BSC = Biological Safety Cabinet

2.4 CDC = Centers for Disease Control and Prevention

2.5 CHP = Chemical Hygiene Plan

2.6 MLB = Microbiology Laboratory Branch

2.7 OEP = Occupant Emergency Plan

2.8 PPE = Personal Protective Equipment

2.9 SHEM = Safety, Health and Environmental Manager

2.10 Restricted Access Alert:

2.10.1 A Restricted Access Alert will be issued when the Branch Chief, Senior Science Advisor, SHEM or Facility Manager have been notified of a microbiological emergency (e.g., a release of anthrax spores) requiring the use of Room D122.

2.11 Restricted Access:

2.11.1 Restricted Access to the Receiving Room D122 will occur when a Restricted Access Alert has been issued. The Branch Chief, Senior Science Advisor or SHEM Manager will notify the Facilities Manager to modify the card reader access to allow entry to authorized individuals. (The MLB Branch Chief maintains the list of authorized individuals).

3.0 HEALTH AND SAFETY:

3.1 To protect the laboratory worker and work environment from possible exposure to *Bacillus anthracis,* the health and safety guidelines provided in SOP MB-01, Biosafety in the Laboratory and in the BMBL (see ref. 15.4) manual must be followed. The manual is available in the laboratory. All laboratory personnel are required to read and familiarize themselves with the sections on Biosafety Level (BSL) 3.

3.2 Laboratory workers must familiarize themselves with this SOP, the laboratory's biosafety spill clean-up procedures (SOP MB-13, Handling Spills of Biohazardous Material), the facility's Chemical Hygiene Plan (CHP) and Occupant Emergency Plan (OEP) prior to performing any laboratory work. Biosafety spill clean-up procedures are posted in the laboratory.

3.3 Medical emergencies are handled according to procedures outlined in the ESC Occupant Emergency Plan (OEP). All emergencies are reported to the SHEM manager. In the event a medical emergency is beyond the scope of the SHEM manager, personnel should call 911 and the security desk at 2800.

3.4 Spills and accidents are handled according to the practices outlined in SOP MB-01, Biosafety in the Laboratory and SOP MB-13, Handling Spills of Biohazardous Material, as well as procedures referenced in the OEP and section 12 of the CHP. In the event a spill occurs when the SHEM Manager is not available, personnel should call 911 and the security desk at 2800.

3.5 All laboratory workers must meet the requirements of the Hazard Communication Program's Employee Training Program, as described in the CHP, section 8.

3.6 In accordance with the CDC/NIH guidelines (see ref. 15.4) the Branch Chief may restrict access to the laboratory as specified under "special practices."

3.7 All employees required to use respirators are participants in the Agency Occupational Medical Surveillance Program and the ESC Respiratory Protection Program. They have been fit tested for the specific respirator, attended respirator use training, receive annual respirator fit testing, have placed documentation of training in the training file, and ensure that their respirators are inspected on a monthly basis as specified in section 4 of the CHP.

3.8 No material suspected or known to be contaminated with biohazardous material (e.g., latex gloves, pipet wrappers, paper towels, etc.) is to be placed in the trash cans. These items are to be placed in a biohazardous waste bag.

4.0 CAUTIONS:

4.1 Lack of use or understanding of this SOP may negatively impact the safety and quality of the microbiological practices used in the laboratory and, hence, the laboratory's mission.

4.2 Failure to use the "STOP/DO NOT ENTER" signs to control access to the laboratory while work is in progress may result in the inadvertent exposure of personnel to biohazardous microorganisms while work is in progress.

4.3 Failure to clean the ultraviolet lamps in the BSC will reduce the lamps' effectiveness. Periodically clean the ultraviolet lamps in the BSC with a lint-free cloth dampened with alcohol.

4.4 Do not enter rooms D122 or D122A when the UV lights are operational.

4.5 Personnel conducting sampling or disinfection of decontamination wastewater that places them at risk for exposure to *Bacillus anthracis* (anthrax) should wear personal protective equipment (PPE), including respiratory devices, protective clothing, and gloves.

5.0 INTERFERENCES:

5.1 Failure to become familiar with and to put into practice the procedures set forth in this SOP will result in analysts who are a danger to themselves, others, and the environment.

5.2 Improper maintenance and/or expired certification may result in failure of the BSC to operate properly. Refer to proper use and maintenance procedures in SOP QC-06, Use and Maintenance of Biological Safety Cabinets.

6.0 PERSONNEL QUALIFICATIONS:

6.1 Personnel qualified to conduct work associated with *Bacillus anthracis* must have CDC Clearance.

6.2 Personnel are required to be knowledgeable of the procedures in this SOP. Documentation of training and familiarization with this SOP can be found in the training file for each employee.

6.3 Each analyst will complete safety re-certification training on at least an annual basis. The facility SHEM manager is responsible for coordinating the training program.

6.4 Key card readers are used to limit access to testing laboratories. Only authorized personnel are permitted to enter.

7.0 SPECIAL APPARATUS AND MATERIALS:

7.1 Biological Safety Cabinet (BSC)

7.2 Autoclave

7.3 Absorbent padding to line BSC prior to work

7.4 Biohazard bags (clear in color, autoclavable) or containers inside and outside of the biological safety cabinets for collection and storage of biohazardous waste.

7.5 When specified, personal protective equipment (PPE) such as latex gloves, latex inner glove/nitrile outer glove combination (for work with risk of exposure to *Bacillus anthracis*), safety glasses, lab coats, disposable tyvek laboratory garments, booties, and respiratory protection (P-100 half-face respirators with HEPA filter cartridges).

7.6 Appropriate signs to identify biohazardous materials and to limit access to laboratories.

7.7 Bleach solutions made fresh as needed. Discard solution at the end of the day.

7.7.1 Bleach solution to be used as a sporicide to treat spore-forming bacteria. Prepare a 1:11.4 dilution of an EPA-registered bleach product (containing at least 50,000-60,000 ppm sodium hypochlorite) at approximately neutral pH in the following manner: 1 part EPA-registered household bleach product containing 50,000-60,000 ppm sodium hypochlorite: 9.4 parts water: 1 part distilled white vinegar (5% acetic acid). Alternatively, a reagent grade solution of sodium hypochlorite adjusted with acetic acid may be used. A 1:11.4 solution of bleach at approximately neutral pH with a contact time of 60 minutes is effective against 5 to 6 logs of *Bacillus subtilis* spores.

7.8 Verify the ppm of available chlorine using the Hach Kit for Total Available Chlorine prior to use.

8.0 INSTRUMENT OR METHOD CALIBRATION: Not applicable

9.0 SAMPLE HANDLING AND STORAGE:

9.1 See Section 10.0 for details on Sample Handling.

10.0 PROCEDURE AND ANALYSIS:

Section I. Procedures for Working in room D122 with Select Agents

10.1 Required PPE. Analysts must wear the following PPE when conducting any work with potential risk of exposure (refer to SOP MB-01, Biosafety in the Laboratory, Section 10.5 for donning and doffing procedures):

10.1.1 Tyvek suits

10.1.2 Double layer of gloves (inner layer-latex, outer layer- nitrile)

10.1.3 Booties if tyvek suits do not have feet

10.1.4 P-100 half-face respirators with HEPA filter cartridges.

Note: HEPA filter cartridges are single use only as specified in Section 4.7.4 of the CHP

10.1.5 Safety glasses

10.2 Additional Procedures.

10.2.1 Sign-In/Out Form. During a Restricted Access Alert, all personnel working in the Receiving Room D122 must sign in and out and on the room D122 Sample Triage Room Sign-In/Out Form, which is posted outside of room D122A.

10.2.2 Buddy System Requirement. At least two analysts with specified PPE will be present in the lab during all work. (Refer to SOP MB-01, Biosafety in the Laboratory, section 10.7 for additional procedures.)

10.2.3 Decontamination of biohazardous waste.

10.2.3.1 Biohazardous waste generated from work conducted in the BSL 3 laboratory will be autoclaved daily using a 3 hour liquid kill cycle for liquid and/or solid waste. Refer to SOP QC-13, Performance Verification of Autoclaves, for verification of autoclave performance and corrective actions.

10.2.3.2 In addition to the maximum registering thermometer and chemical strip, a biological spore ampule will be included with each run to provide additional quality control.

10.2.3.3 Incubate the ampule for 48±2 hours at 55-60ºC.

10.2.3.4 If results pass all quality control measures, the autoclaved trash may be discarded with general laboratory waste. If results do not pass all quality control measures, repeat steps 10.2.3.1 thru 10.2.3.3.

10.3 BSC work and cleanup.

10.3.1 Prior to working in the BSC, line the bottom surface with two layers of absorbent padding. Place a small autoclavable (e.g., Nalgene) container in the BSC for all waste materials.

10.3.2 Following use, decontaminate inner surfaces of the BSC with Lysol IC or 70% ethanol. Decontaminate by spraying the surface of the BSC, allowing the solution to sit for up to 10 minutes and then wiping the solution using absorbent padding or disposable paper towels. Turn on the ultraviolet (UV) light in the BSC and leave it on overnight (minimum of 15 hours).

10.4 Transport/storage of environmental samples, cultures, slides.

10.4.1 Secondary containment (Nalgene tub) will be used for transport and storage of environmental samples, cultures, slides etc. within room D122.

10.4.2 Environmental samples, cultures, slides, etc. may not be removed from the room D122 laboratory unless enclosed in a sealed, secure, non-breakable container (e.g., Nalgene polyethylene, High Density Polyethylene, Carboy) with the exterior decontaminated with the bleach solution.

10.5 Treatment of Spills

10.5.1 If a spill occurs in the BSC or on the stainless steel benchtops in room D122 where absorbent padding has not been placed, use the bleach solution to pre-clean the surface where the spill occurred. Autoclave any material (e.g., absorbent padding, disposable paper towels) used to pre-clean a biohazardous spill of *Bacillus anthracis*.

Note: Do not allow the bleach solution to remain on stainless steel for one hour as specified in Section 7.7.1. Flush the BSC after five minutes contact time as specified in Section 10.5.2

10.5.2 Flush the BSC or benchtop with water, 70% ethanol or Lysol IC, to remove any excess sodium hypochlorite if absorbent padding was not present on the surface prior to the spill. Once the spill has been pre-cleaned and the liquid wiped up, turn on the UV lights to the BSC or the room to decontaminate the area of the spill for a minimum of 15 hours (overnight).

10.5.3 If absorbent padding was present prior to work, place the contaminated absorbent padding in the waste container in the BSC, wipe down the BSC or benchtop surface with 70% ethanol or

Lysol IC, replace the absorbent padding and resume work. Upon completion of work in the BSC turn on the UV lights to the BSC or the room to decontaminate the area of the spill for a minimum of 15 hours (overnight).

10.5.3 If a spill occurs outside of the BSC (e.g., on the floor), contact the SHEM Manager (or the security desk at extension 2800) and the Branch Chief or Senior Science Advisor by telephone from the laboratory.

Section II. Operation and Maintenance of the UV Lights in room D122.

10.1. The UV lights will be used under the following conditions:

10.1.1 Uncontrolled or large microbiological spill of non-select agents outside of the BSC

10.1.2 Following an event when select agents (e.g., *Bacillus anthracis*) were received and processed.

10.1.3 Following routine use of the room with non-select agents (all other microbiological organism).

10.2 To Turn UV Lights ON:

10.2.1 Upon leaving the room, verify there are no personnel remaining in the room.

10.2.2 Ensure all doors are securely closed.

10.2.3 Set time switch(s) for desired duration of UV light operation (minimum of 15 hours for select agent procedures)

10.2.4 Once time switch is set and lights are turned on, do not open any doors without first turning the UV Lights OFF.

10.2.5 Post UV light sign on the door.

10.2.6 If door is opened with UV Lights operational, the Shunt Trip Breaker will trip. If the shunt trip breaker trips see Section II, 10.4 below.

10.3 To Turn UV Lights OFF

10.3.1 Turn time switch to the OFF position prior to entering the room with UV lights in operation.

10.3.3 UV lights are now deactivated and room may be entered.

10.3.4 Remove the UV light sign from the outside of the door.

10.4 What To Do if Shunt Trip Circuit Breaker is Tripped

10.4.1 On the outside wall of room D122A above the door, there is a circuit breaker enclosure containing a Shunt Trip circuit breaker that supplies power to the UV Lights. This system is wired so that the Shunt Trip Breaker will shut down power to the UV Lights if the door is opened while the lights are in operation. This will prevent personnel from entering the room while the UV lights are on.

10.4.2 If the shunt trip breaker is tripped by opening the door when the lights are on, follow these steps:

10.4.2.1 Close the door. The UV lights will remain off.

10.4.2.2 Turn the time switch selector to the “physical” OFF position.

10.4.2.3 To reset shunt trip breaker, pull the toggle all the way down to the OFF position and then push it all the way up to the ON position. The toggle should stay up in the ON position and the lights are now once again operational.

10.4.3 If the shunt trip does not reset, call the Facilities Helpline (4357) for further assistance.

Section III. Decontamination of the Holding Tank

10.1 Wastewater generated from room D122 (e.g., sink, rinsate from pre-cleaning etc.) flows to a High Density Polyethylene (HDPE) 200 gallon holding tank in room D123. In order to have sufficient space for decontamination of the wastewater, do not allow the holding tank to fill above the 100 gallon mark. Analysts should

verify wastewater quantity in the holding tank on a daily basis to determine the need for decontamination.

10.2 Wastewater generated from room D122 during a Restricted Access Alert may not be discharged into the neutralization tank at the ESC until it is treated in accordance with the guidelines specified in the National Response Team (NRT) Technical Assistance for Anthrax Response (see attached, Appendix E). Post decontamination sampling must ensure that no viable spores are present in the wastewater prior to discharge. Documentation of post-decontamination sampling must be sent to the SHEM and Facility Manager. Only the SHEM or Facility Manager may approve post-decontamination discharge of the holding tank in room D123.

10.3 Decontamination of the holding tank will be performed by appropriate personnel under the direction of the SHEM manager.

10.4 See Appendix E of NRT procedures for disinfection of holding tank.

10.5 Record decontamination of holding tank on form 16.2: Decontamination of Holding Tank (Room D123) Log.

Section IV. Sampling of the Wastewater in the Holding Tank

10.1 Sampling of the decontamination wastewater should be conducted in accordance with the National Response Team Assistance for Anthrax Response. See reference 15.2. Remove decontaminated wastewater from room D123 using secondary containment such as Nalgene, polyethylene, High Density Polyethylene, or Carboy containers.

Section V. Wastewater Sample Analysis

10.1 Using your environmental sampling device, mix the liquid in the holding tank in room D123 for a few minutes.

10.2 Using an environmental sampling device (e.g., Coliwasa, Drum Thief etc.), remove 2 to 5% of the total volume of liquid in the holding tank and place in a sterile carboy container. Cap the carboy container. Use secondary containment and transport to room D122.

10.3 Prior to filtering the liquid, mix by swirling. Filter 150 mL through a sterile 0.45 µm filter unit. Rinse the filter unit with FTM w/ 0.1% sodium thiosulfate to

neutralize the sodium hypochlorite followed by a rinse with 0.85% saline or sterile deionized water. More than one filter unit may be used.

10.4 Place the filter(s) onto Trypic Soy Agar (TSA) or TSA with Blood Agar, 1 filter per plate. Incubate the plate(s) at 36±1°C for a minimum of 48±2 hours.

10.5 After the incubation period, examine the filters(s) for growth or no growth.

10.5.1 If no growth observed: the sample is free of viable microorganisms. Submit memo of the results to the SHEM and Facility Manager at the ESC, and the Branch Chief of the Microbiology Laboratory Branch for request of wastewater release from the holding tank.

10.5.2 If growth observed: Gram stain the growth in room D122. If gram positive rods are present, streak isolate onto TSA and incubate for 18-24 hours. If the plate appears to be a pure culture then go to VITEK for confirmation of the organism. (Refer to QC-22, VITEK 2 Compact: Use, Maintenance and Quality Control Procedures)

10.5.3 If *Bacillus anthracis* is identified via VITEK, decontaminate the wastewater again and sample 24 hours later. Repeat steps 10.1 through 10.5.

10.6 Once the wastewater has been cleared for release by the SHEM or Facility Manager, open the release valves on the holding tank until the majority of decontaminated wastewater is removed.

NOTE: A small amount of liquid will always remain at the bottom of the holding tank.

11.0 DATA ANALYSIS/CALCULATIONS: None

12.0 DATA MANAGEMENT/RECORDS MANAGEMENT:

12.1 Data will be recorded promptly, legibly, and in indelible ink on the appropriate forms. Completed forms are archived in notebooks kept in secure file cabinets in file room D217. Only authorized personnel have access to the secure files. Archived data is subject to OPP's official retention schedule contained in SOP ADM-03, Records and Archives.

13.0 QUALITY CONTROL: None

14.0 NONCONFORMANCE AND CORRECTIVE ACTION:

14.1 Strict adherence to the biosafety practices is required. Nonconformance will result in notification, retraining, or disciplinary action of laboratory employees.

15.0 REFERENCES:

15.1 Fleming, D.O. and Hunt, D.L. eds. 2000. Biological Safety: Principles and Practices. ASM Press, Washington, D.C.

15.2 National Response Team. 2005. Technical Assistance for Anthrax Response. Interim-Final Draft, Appendix E: attached.

15.3 Richmond, J.Y. and McKinney, R.W. eds. 1999. Biosafety in Microbiological and Biomedical Laboratories (BMBL). HHS Publication No. (CDC) 93-8395.U.S. Government Printing Office, Washington, D.C.

15.4 Additional Requirements for Facilities Transferring or Receiving Select Agents, 42 CFR Part 72.6.

15.5 Operation and Maintenance Manuals: Volume 2-5. Anthrax Lab Modifications. Ft. Meade, MD. MKM Engineers, Inc.

15.6 QAPP 2003-01-D122-Wastewater Final Summary Report, OPP Microbiology Laboratory

16.0 FORMS AND DATA SHEETS:

16.1 Room D122 Sample Triage Room Sign-In/Out Form

16.2 Decontamination of Holding Tank (Room D123) Log

Room D122 Sample Triage Room Sign-In/Out Form

OPP Microbiology Laboratory

Date	Name	Nature of Work	Time In	Time Out

Decontamination of Holding Tank (Room D123) Log

OPP Microbiology Laboratory

INFORMATION				
Date/Initials	Amount of wastewater in the holding tank (estimated)	Amount of Bleach/Vinegar** solution added per NRT guidelines*	Date cleared for discharge/Initials	Date of Discharge/Initials

*Refer to attached NRT guidelines for instructions on decontamination procedures.
**Acetic Acid may be substituted for Household Vinegar. Use Hach Kit for Total Available Chlorine to verify ppm of solution.